



Nitrogen uptake capacity of European beech (*Fagus sylvatica* L.) only partially depends on tree age

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Abstract

Key message On calcareous soil, European beech roots prefer organic nitrogen, but only arginine and not glutamine or inorganic nitrogen.

Abstract Nitrogen (N) acquisition is a major factor determining the processes and mechanisms involved in tree productivity, development, and competitiveness. However, only few studies have investigated changes in N capturing with tree age. We conducted ¹⁵N incubation experiments to quantify inorganic (i.e. ammonium and nitrate) and organic (i.e. glutamine-N and arginine-N) net N acquisition capacity of beech trees of five age classes. Our results showed no general pattern, but that net N uptake capacity was rather N source-specific. Inorganic and glutamine-N uptake did not differ between age classes at all. Arginine-N uptake was highest in the youngest and oldest stands reflecting a high N demand by seedlings for root foraging and biomass production despite low internal N storage capacities and by older trees for storage and reproduction. Organic N was preferred over inorganic N regardless of tree age. Overall, our study shows the high significance of organic N sources for N acquisition in beech trees.

Keywords Ammonium · Nitrate · Glutamine · Arginine · Nitrogen uptake

Introduction

The capacity to capture resources, particularly nitrogen (N), plays an important role in achieving maximum growth in long-living woody species (Körner, 2003; Millard et al. 2007; Millard and Grelet, 2010). Particularly in deciduous trees, growth depends on both external uptake of N from the soil, but also internal N pools (Millard, 1996; Dyckmans and Flessa, 2001) and is regulated by the internal storage

capacity (Tagliavini et al. 1997; Rennenberg et al. 2010). For example, more N is taken up by seedlings at the beginning of the growing season because N demand is high and internal N storage capacity is low due to a restricted storage capacity and the use of internal resources for leaf development (Fotelli et al. 2004; Simon et al. 2011). Overall, the resource capturing capacity of a tree is regulated by its size which in turn is linked to its age via the accumulation during past growth (e.g. Millard and Grelet, 2010; Aakala et al. 2013; De Groote et al. 2018). However, studies on age-related effects are scarce and mostly based on the stand level including tree productivity (Olsen et al. 1998; Portsmouth et al. 2005; De Groote et al. 2018) and/or soil properties (Staska et al. 2014; Wang et al. 2017).

N acquisition is a key aspect in understanding the processes and mechanisms leading to high productivity and competitiveness, but only few studies have investigated shifts in N capturing strategies (i.e. via external acquisition or internal remobilisation of N) with increasing age at the individual tree level (Simon et al. 2011; Sun et al. 2016). Thus, the influence of tree age on N acquisition from the soil is still not fully understood (Liu et al. 2018). For example, with increasing tree age, root morphology and physiology

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are likely to change as younger individuals require and allocate more N to increase root length and surface area for better root foraging (Saulnier and Reekie, 1995; Ryan et al. 1997; Liu et al. 2018). Furthermore, with increasing age more N is stored in the heartwood of trees and their litter, thus overall soil N availability might be reduced over time during forest development (Ryan et al. 1995).

This study builds on previous work investigating the uptake capacity of seedlings and adult individuals of *Fagus sylvatica* for inorganic and organic N over the growing season (Simon et al. 2011). Here, we hypothesise that the acquisition of inorganic and organic N resources from the soil decreases with tree age as tree internal storage capacity and, thus, remobilisation from storage pools rise (Simon et al. 2011). We investigated five forest stands differing in age at a site on calcareous soil. We chose European beech as a model species, because it represents the potential natural vegetation in moist to moderately dry areas of the submountainous altitude range in Central Europe (Ellenberg and Leuschner, 2014) and has been favoured by forest practitioners (Petritan et al. 2009; Simon et al. 2011) despite its sensitivity to drought (Geßler et al. 2007; Houston et al. 2016).

Materials and methods

Field site characteristics and experimental design

The study was conducted in beech-dominated forest stands of different age classes at the “Schönberg” (department “Leisacker” and “Baumgarten”) in Freiburg im Breisgau in southern Germany (47°57'N, 7°48'O, 645 m above sea level). The soil is a rendzina on calcareous parent rock with a high pH and a low water holding capacity (Körner, 2006). Hädrich and Stahr, 2001 reported 0.79–1.05% total soil N in the Ah horizon (ranging from 0 to 15 cm depth) which is similar to that of other beech forests on calcareous soil (see Pena et al. 2010: 0.8% total soil N). Mean annual temperature and precipitation were 11.4 °C and 934 mm, respectively, at the weather station Freiburg (#1443, 48.0232, 7.8344; 236 m a. s. l.; 1981–2010, Deutscher Wetterdienst

DWD). Sites were set up in stands of different European beech (*Fagus sylvatica* L.) age classes with three replicate plots per age class. Per plot and age class, ¹⁵N incubation experiments were performed on 5–8 individual beech trees (with a total of 15–24 individuals per age class). At the sampling time, the five age classes included e1—5 to 10 years, e2—12 to 25 years, e3—23 to 31 years, e5—43 to 65 years, and e12—85 to 130 years old beech trees (see Table 1). Forests stands were beech-dominated, except for age class e2 where *Fagus sylvatica* was mixed with few individuals of *Acer pseudoplatanus* L., *Prunus avium* L., and *Fraxinus excelsior* L. of similar age. For each plot, soil samples were taken from top soil (max. 10 cm depth) at five different locations and pooled. Subsamples were sieved, dried at 60 °C for 72 h. Total N % was quantified using an elemental analyser (see below).

¹⁵N uptake experiments

¹⁵N uptake experiments were conducted in autumn before leaf senescence. To quantify inorganic and organic net N uptake capacity by mycorrhizal fine roots, we used the ¹⁵N enrichment technique described by Gessler et al. (1998) as modified by Simon et al. (2010a). For this purpose, fine roots still attached to the individual trees were carefully dug out and cleaned of adherent soil material using tap water. Five root tips (c. 40–60 mg each) were incubated individually in 4 ml of one of five artificial soil solutions for 2 h. The artificial soil solution was modelled as previously reported for another field site on calcareous soil (Gessler et al. 2005; Simon et al. 2011) and contained four different N sources one of which was ¹⁵N/¹³C-labelled (i.e. ammonium, nitrate, glutamine, or arginine; all with > 97% enrichment) or without label (i.e. to account for natural abundance). Overall, the solution contained 90 μM CaCl₂ * 2 H₂O, 70 μM MgCl₂ * 6 H₂O, 50 μM KCl, 24 μM MnCl₂ * 4 H₂O, 20 μM NaCl, 10 μM AlCl₃, 7 μM FeSO₄ * 7 H₂O, 6 μM K₂HPO₄, 100 μM KNO₃, and 1 μM NH₄Cl, as well as the amino acids glutamine (Gln) and arginine (Arg) (both 25 μM). Gln and Arg were chosen as amino acids because they are the most abundant amino acids in European beech roots (Stoelken

Table 1 Field site description. Age class, age range (years), tree diameter at breast height (DBH, cm), presence of other tree species, total N in soil (%), and total N in the fine roots (%)

Class	Age	DBH	In plots mixed with	N in soil	N in fine roots
e1	5–10	2.9 ± 1.0	n.a	0.25 ± 0.01 ^a	0.95 ± 0.20 ^a
e2	12–25	9.0 ± 3.1	<i>Acer pseudoplatanus</i> , <i>Prunus avium</i> , <i>Fraxinus excelsior</i>	0.42 ± 0.01 ^b	0.87 ± 0.16 ^a
e3	23–31	12.7 ± 2.3	n.a	0.40 ± 0.01 ^b	1.16 ± 0.21 ^{bc}
e5	43–65	23.0 ± 3.8	n.a	0.50 ± 0.03 ^c	1.27 ± 0.25 ^b
e12	85–130	43.0 ± 10.0	Very few <i>Abies alba</i>	0.47 ± 0.01 ^c	1.01 ± 0.17 ^{ac}

Different letters indicate significant differences between age classes ($p \leq 0.050$)

n.a. not applicable

et al. 2010) and also represent dominant amino acids in forest soil (Inselsbacher et al. 2011). The amino acid concentration used was within the range of estimates previously reported (Inselsbacher et al. 2011). Incubation experiments were conducted between 10 am and 2 pm to avoid diurnal variation of N uptake (Gessler et al. 2002). After 2 h of incubation, the submerged root tips were cut off, washed in 0.5 μM CaCl_2 to remove excess ^{15}N on the surface and dried with cellulose tissue. After determining the fresh weight, the root samples were oven-dried (48 h at 60 °C) and the dry weight was recorded. Dry root material was ground to a fine homogenous powder using a ball mill and prepared for isotope ratio mass spectrometry.

Quantification of ^{15}N , ^{13}C , and total N and C amounts in fine roots

For the quantification of ^{15}N , ^{13}C , as well as total N and C amounts in the fine roots of beech trees of different age classes, aliquots of 1.2–2.5 mg homogenous root powder were weighed into tin capsules (4–6 mm, IVA Analysentechnik, Meerbusch, Germany) and analysed using an elemental analyser (NA2500, CE Instruments, Milan, Italy) coupled via a ConFlo II interface to an isotope ratio mass spectrometer (Delta Plus, Thermo Finnigan MAT GmbH, Bremen, Germany). Glutamic acid was analysed as working standard after every 12th sample to detect potential instrument drift over time. This standard was calibrated against the primary standards USGS 25 ($\delta^{15}\text{N}_{\text{air}} = -30.4$) and USGS 41 ($\delta^{15}\text{N}_{\text{air}} = 47.600$) for ^{15}N and USGS 40 ($\delta^{13}\text{C}_{\text{PDB}} = -26.39$) and USGS 41 ($\delta^{13}\text{C}_{\text{PDB}} = 37.63$) for ^{13}C . Inorganic and organic net N uptake capacity was calculated based on the incorporation of ^{15}N into the fresh root biomass according to the equation reported by Gessler et al. (1998): net N uptake capacity = $((^{15}\text{N}_1 - ^{15}\text{N}_n) * N_{\text{tot}} * dw * 10^5) / (MW * fw * t)$, where $^{15}\text{N}_1$ and $^{15}\text{N}_n$ are the atom% of ^{15}N in labelled (N_1) and non-labelled (N_n , natural abundance) roots, respectively, N_{tot} is total N percentage, "MW is the molecular weight of ^{15}N and t represents the incubation time (120 min)". ^{13}C net uptake capacity of Gln and Arg was generally lower compared to those based on ^{15}N suggesting (1) a potential degradation of Gln and Arg during the incubation in the artificial soil solution and/or on the root surface and/or (2) the respiration of amino acid-derived carbon inside the roots (Simon et al. 2011).

Statistical analyses

Data were tested for normality (Shapiro–Wilk test) and equal variances (Brown–Forsythe test). Differences in soil N levels between age classes were tested by one-way ANOVA followed by the post hoc Holm–Sidak test. Kruskal–Wallis one-way ANOVA on Ranks followed by post hoc Dunn's test was

performed to test for (1) the effect of age class on each N source (i.e. ammonium, nitrate, glutamine-N, and arginine-N), (2) the preference for certain N sources within each age class, and (3) total soil N and C in the fine roots between age classes. Significant differences were tested at $p \leq 0.050$ using Sigmaplot 13.0 (Systat Software Inc., San Jose, CA, USA).

Results and discussion

Total soil N increases with tree age

Soil N levels might decline with stand age because of the accumulation of N in tree biomass and immobilisation during wood decomposition (Murty et al. 1996; Gover et al. 1996; Ryan et al. 1997). However, studies investigating the mineralisation rates of nutrients in forest chronosequences found no general pattern (Ryan et al. 1997). In our study, total soil N levels significantly increased with stand age, i.e. age classes e12 and e5 > e3 and e2 > e1 ($p \leq 0.003$), and varied from 0.25% in the youngest to 0.47% in the oldest age class (Table 1). These levels are between those measured at the research stations with beech forest on calcareous soil in Tuttlingen, Germany (on average 0.80 ± 0.01 total N % in the top soil; Pena et al. 2010) and acidic soil in the Höglwald, Germany (on average 0.23 ± 0.05 total N % in the top soil; Stoelken et al. 2010). Total N levels in the soil did not reflect the levels in the fine roots which is likely due the unavailability of some N pools in the soil for root acquisition, for example N immobilised by soil microbes (Simon et al. 2017). In our study, total N levels in fine roots were higher for the middle-aged trees compared to the youngest and oldest (i.e. age class e3 > e1 and e2, as well as e5 > e1, e2 and e12; $p \leq 0.045$; Table 1). Total C levels of the fine roots ranged from 44.68 ± 5.41 total C % in the youngest to 42.21 ± 4.28 total C % in the oldest forest stand and did not differ between age classes. High N levels are required for root growth and development to enhance the foraging capacities, especially in seedlings (Chapin et al. 1994; Saunier and Reekie; Liu et al. 2018), further N is also required in mature trees for reproduction (Chapin et al. 1994; Saunier and Reekie, 1995). Soil N levels between young and adult beech trees were not different in the study by Simon et al. (2011) which could be explained by age differences between the studies (i.e. seedling age was comparable to that in our study, whereas the age of the adult trees was between e5 and e12).

Patterns of N acquisition with increasing tree age are source-specific

The comparison of inorganic and organic N uptake capacity among the five investigated age classes in our study showed no general pattern, but was rather N source-specific.

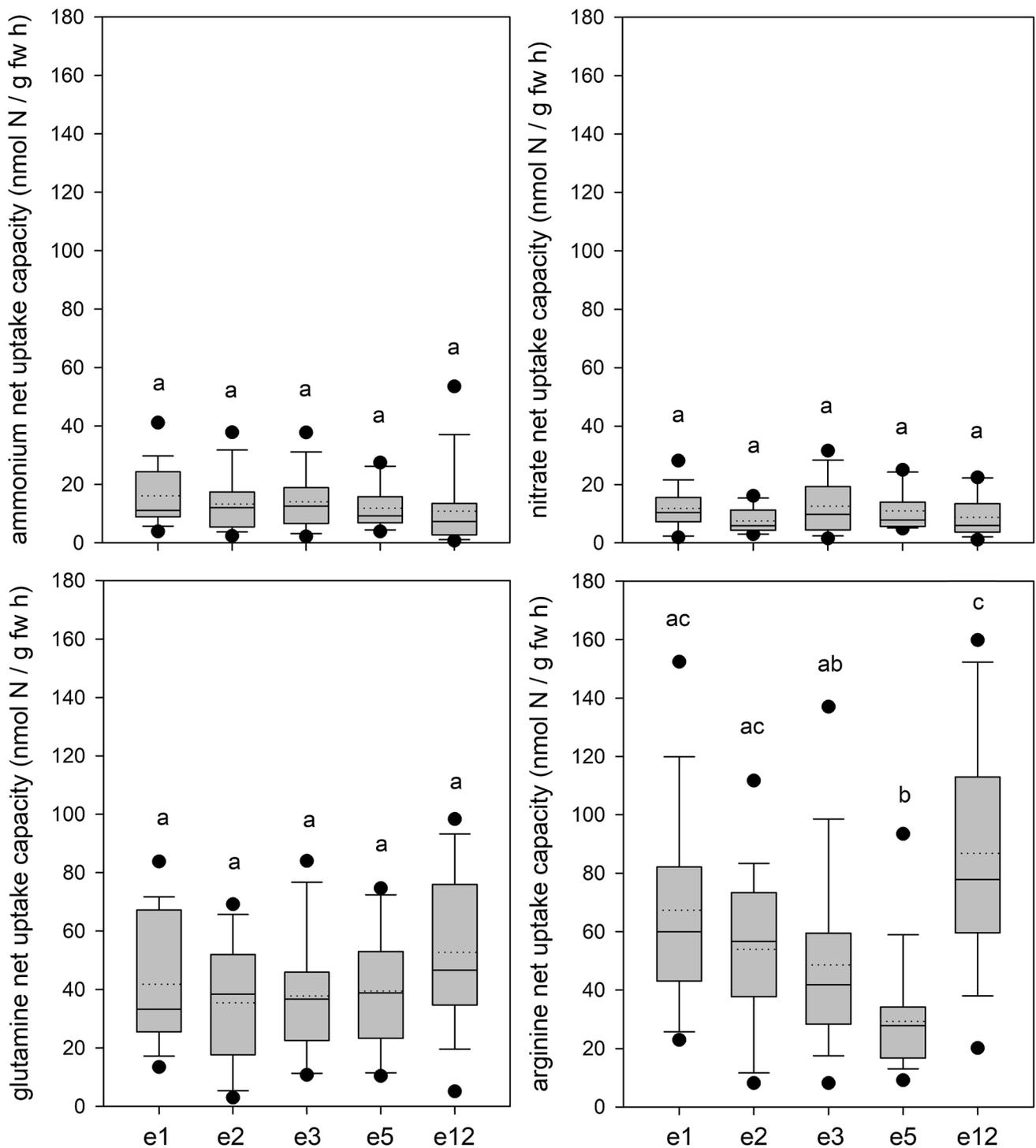


Fig. 1 Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity ($\mu\text{mol N/g fw h}$) of the fine roots of European beech at five different age classes. e1=5–10 years, e2=12–25 years, e3=23–31 years, e5=43–65 years, and e12=85–130 years. Box

plots show mean (dotted line) and median (straight line). Different letters indicate significant differences among age classes within a specific N source ($p < 0.050$)

Ammonium, nitrate, and glutamine-N net uptake capacity did not differ at all among age classes, whereas arginine-N net uptake capacity was higher in the two youngest and the oldest stands compared to the middle-aged stands: e1, e2,

plots show mean (dotted line) and median (straight line). Different letters indicate significant differences among age classes within a specific N source ($p < 0.050$)

e12 > e5 ($p \leq 0.049$), and e12 > e3 ($p = 0.012$; Fig. 1, Supplemental Table 1). These results differ from those in the two other studies that looked at changes in inorganic and organic N acquisition in trees with age. Liu et al. (2018) studied N

uptake in *Hevea brasiliensis* (rubber tree) plantations and found that with increasing tree age glycine uptake increased, whereas ammonium uptake decreased. Simon et al. (2011) compared N acquisition of seedlings and adult beech trees over the growing season: both net nitrate and glutamine-N uptake capacity were higher in mature beech compared to seedlings, whereas net ammonium uptake capacity did not differ (Simon et al. 2011). However, the studied trees of different age in Simon et al. (2011) were within the same stand indicating seasonal avoidance of potential competition between seedlings and mature trees, whereas in the current study age classes were in strictly separated forest stands. Furthermore, differences in the uptake of specific amino acids might be related to their abundance in the soil (Simon et al. 2017). Especially positively charged amino acids, such as arginine, are likely to dominate soil diffuse N fluxes when released during the degradation of microbial and/or plant tissue which could then affect the expression of the relevant arginine transporters in plants (Inselsbacher and Wanek, 2021), and thus their N uptake capacity. Mycorrhiza might have an additional effect on a tree's N acquisition strategy as mycorrhizal community composition might change with tree age. However, a previous study on mycorrhiza and beech seedlings found that organic N uptake occurs regardless of mycorrhization (Stoelken et al. 2010). Furthermore, Leberecht et al. (2016) showed that ectomycorrhiza preferred ammonium, whereas beech preferred nitrate, thus avoiding competition for the same inorganic N sources. How this plays out for organic N sources and/or shifts with increasing tree age is still unknown.

The N conservation strategy of trees is regulated by its N uptake and storage capacities as well as the availability of N in the soil (Reich et al. 1997; Sun et al. 2016; Simon et al. 2017). In our study, arginine-N uptake capacity was higher in youngest and oldest trees, confirming our initial hypothesis that younger trees take up more N from the soil due to their low internal N storage capacity (e.g. Millard and Grelet, 2010; Simon et al. 2011; Sun et al. 2016) most likely via a higher uptake capacity for N. The higher arginine-N acquisition by beech trees in the oldest stand compared to the middle-aged trees contradicts our initial hypothesis that older trees take up less N from the soil as they rely more on internal N remobilisation from storage (e.g. Millard and Grelet, 2010; Simon et al. 2011). However, effective N acquisition from the soil might differ from the capacity of roots to take up N and is likely related to the relative abundance of different N forms (Inselsbacher and Wanek, 2021). Furthermore, the amino acids used in this study have different functions within plants: glutamine is the main amino acid transported in beech, whereas arginine is a storage amino acid (Millard and Grelet, 2010; Babst and Coleman, 2018). Thus, considering the abundance of specific N forms in the soil as well as the shift in a tree's demand for N over its lifetime,

this might also lead to changes in N acquisition strategy. For example, at the seedling stage, trees require more N to increase root foraging for growth and biomass production and the use of enhanced storage capacity, whereas mature trees need N for reproduction (Chapin et al. 1994; Saunier and Reekie, 1995) with a potentially declining nitrogen use efficiency (Ryan et al. 1997).

General preference for organic over inorganic N sources over all age classes

Comparing inorganic and organic N uptake capacity within each age class, net uptake capacity of organic N was generally higher than that of inorganic N ($p \leq 0.019$). These results confirm previous studies quantifying inorganic and organic net N uptake capacity of adult beech and/or beech seedlings under controlled conditions and in the field that were incubated with an artificial N solution of the same composition: beech trees generally preferred organic over inorganic N, and nitrate over ammonium (see Supplemental Table 1). These patterns were found regardless of age (i.e. mature beech vs. seedlings, Simon et al. 2011), species (i.e. Simon et al. 2010; Dong et al. 2016; Li et al. 2016), abiotic (e.g. light availability; Simon et al. 2014) as well as biotic factors (e.g. competition with sycamore maple; Simon et al. 2010; Li et al. 2016).

In conclusion, tree age or rather its developmental stage does have some effect on the acquisition of inorganic and organic N sources from the soil depending on plant internal factors, such as its N demand and internal storage capacities, but also abiotic factors such as the abundance of specific N sources which in turn might regulate the expression of genes encoding N transporters.

Author contribution statement JS and HR conceived and designed the study. JS and SB conducted the ^{15}N uptake experiments at the field sites. SB prepared the samples for IRMS analysis and performed pre-statistical data processing. JS statistically analysed the data and wrote the initial draft of the manuscript. All authors contributed to manuscript revisions and approved the final manuscript.

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Data availability Data will be available from the Dryad Digital Repository upon acceptance of the manuscript.

Code availability Not applicable.

Declarations

Conflict of interest None declared.

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